Heavy Metal–Nucleotide Interactions. 14. Raman Difference Spectrophotometric Studies of Competitive Reactions in Mixtures of Four Nucleotides with the Electrophiles Methylmercury(II) Perchlorate, *cis*-Dimethylgold(III) Perchlorate, Dichloroethylenediaminepalladium(II), *trans*-Dichlorodiamminepalladium(II), *cis*-Dichlorodiammineplatinum(II), *trans*-Dichlorodiammineplatinum(II), Tetra-μ-acetato-dirhodium(II), and Aquopentaamminecobalt(III) Perchlorate. Factors Governing Selectivity in the Binding Reactions[1,2]

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Abstract: Competitive reactions of each of the electrophiles H_3CHg^{II}, *cis*-$(H_3N)_2Pt^{II}$, *trans*-$(H_3N)_2Pt^{II}$, $enPd^{II}$ (en = ethylenediamine), *trans*-$(H_3N)_2Pd^{II}$, *cis*-$(H_3C)_2Au^{III}$, $Rh_2(OOCCH_3)_4$, and $(H_3N)_5Co^{III}$ with a mixture of 20 mM 5′-GMP, 20 mM 5′-CMP, 30 mM 5′-AMP, and 30 mM 5′-UMP have been studied at pH 7, 25°C, using Raman perturbation difference spectroscopy to establish whether there is selectivity in the metal binding to the nuclei acids. Methylmercury(II) shows high selectivity for attack at N–H bonds and binds to UMP N(3) when $0 < r_t$ (total metal:total phosphate) ≤ 0.3. For $0.3 < r_t \leq 0.8$ binding is at N(1) of GMP, and when $r_t > 0.7$ reaction also occurs with CMP, N(3). Both *cis*- and and *trans*-$(H_3N)Pt^{II}$ react with GMP at low r_t values, but coordination is at N(7) and no proton loss from the ligand occurs. The cis isomer is unique in that it exhibits complete selectivity for the two purines, GMP and AMP, when $0 > r_t \leq 0.3$. The trans isomer shows less affinity for AMP. Intermediate behavior is exhibited by $enPd^{II}$, *trans*-$(H_3N)_2Pd^{II}$, and *cis*-$(H_3C)_2Au^{III}$ which react with both purines and pyrimidines and both at ring nitrogens and N–H bonds. Reaction is most extensive with *trans*-$(H_3N)_2Pd^{II}$, least with *cis*-$(H_3C)_2Au^{III}$. Reactions of the diammineplatinum(II) electrophiles at low r_t values are kinetically controlled. As substitutions at the metal center become faster, the product distributions shift toward that observed for H_3CHg^{II} where thermodynamic control obtains. The rhodium(II) acetate dimer behaves entirely differently from the other second- and third-row transition metals and causes almost no perturbation of the nucleotide vibrations upon reaction. The presence of two intense bands involving Rh–Rh stretching shows that the cluster remains intact. The first row transition metal electrophile $(H_3N)_5Co^{III}$ at $r_t = 0.2$ gives no measurable perturbation of the base or phosphate vibrations and appears to form outer-sphere complexes.

Introduction

During the past decade, there have been numerous studies on the interaction of metal ions with nucleic acid constituents.[3] Most have involved one or the other of two procedures. Either some technique, e.g., UV spectrophotometric or potentiometric, is used to determine the equilibrium constant(s) for the interaction of a metal with one nucleoside or nucleotide, or a compound is isolated from a solution containing a metal ion and a single nucleoside or nucleotide. In the latter case, an attempt is made to establish the nature of the coordination in the solid complex, often by infrared or other spectroscopic techniques but more recently in a number of cases by a complete X-ray crystal and molecular structure determination.[4,5] Caution must be exercised because the characterization of a solid compound isolated from a solution may or may not give information about the principal species in the solution, since the least soluble complex may not be the major species. Exceptions to these approaches are the NMR line-broadening studies with paramagentic ions that are used to obtain binding site information with aqueous solutions.[6]

The advantage of solution studies is that they can give, ideally, a quantitative description of the extent of reaction in solution. Because, in practice, the determination of stability constants has proved to be difficult, there have been few studies comparing the stability of complexes of different nucleosides with a particular metal ion.[3a] It has been suggested[3b] that the nucleoside bases can frequently be placed in the following order of relative stabilities of their metal complexes: G > A, C > U, T. In reality, this selectivity is based largely on the behavior of the labile first row transition metal ion Cu^{2+}. In aqueous solution, *cis*-β-$[Co(trien)Cl_2]^+$ was found to exhibit the following order of binding affinity: dT > dG > dC >> dA. Recently stability measurements have been extended to ternary systems, e.g., to the study of mixed

(1) Work supported by Public Health Service Grant AM-16101 from the National Institute for Arthritis, Metabolism, and Digestive Diseases and by National Science Foundation Grant CHE 76-18591.

(2) Previous article in this series: A. J. Canty and R. S. Tobias, *Inorg. Chem.*, **18**, 413 (1979).

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(5) For a review see D. J. Hodgson, *Prog. Inorg. Chem.*, **23**, 211 (1977).

(6) See the discussion and references in G. L. Eichhorn, N. A. Berger, J. J. Butzow, P. Clark, J. M. Rifkind, Y. A. Shin, and E. Tarien, *Adv. Chem. Ser.*, **100**, 135 (1971).

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complexes of a nucleotide, bipyridyl, and a metal ion.[7] Nonaqueous systems will not be considered in this paper, since there are fundamental differences between these and aqueous solutions.

The disadvantage of solution studies is that they usually give no direct structural information. Recourse normally must be made to a comparison of reactions of the nucleoside and a modified nucleoside, usually one blocked by methylation at different positions. However, the methyl derivaties can be regarded themselves as complexes of the methyl cation, an extremely strong electrophile.[8] Consequently, they are poor models for reaction of the nucleoside itself.

In this paper, we have used a competitive reaction technique based on Raman perturbation difference spectrophotometry to study directly the reactions of several metal electrophiles with mixtures of four nucleotides in order to determine the preferred reactions in aqueous solution and the factors governing the specificity, the nucleotide(s) which react, and the site(s) at which the electrophile binds can be determined. The metals were chosen from among those that are known to interact with nucleic acid constituents or are iososteric with such species. The four mononucleotide mixtures serve as models for certain reactions with polynucleotides, particularly in the denatured form where conformational effects are removed. As such, they are necessary to provide information for the interpretation of comparable measurements with the native polynucleotides. The direct extrapolation of these results to polynucleotides[9] must, however, be approached with caution, since phosphate binding will tend to be enhanced in the model, and the blocking effect of the secondary structure of polynucleotides is absent.

Experimental Section

Metal Complexes. Solutions containing H_3CHg^{II} as $[CH_3HgOH_2]^+$-ClO_4^- were prepared from CH_3HgI obtained from Alfa-Ventron, Danvers, Mass., and aqueous $AgClO_4$ as described previously.[10] Solutions containing *cis*-$(CH_3)_2Au^{III}$ as the aquo cation *cis*-$[(CH_3)_2Au(OH_2)_2]^+$-ClO_4^- were prepared in a similar way from $[(CH_3)_2AuI]_2$ as has been described.[11] Synthesis of *trans*-$[PdCl_2(NH_3)_2]$ was by literature methods.[12] Anal. Calcd for $H_6N_2Cl_2Pd$: Cl, 33.5; Pd, 50.3. Found: Cl, 33.7; Pd, 50.3. The infrared spectrum (200–4000 cm^{-1}) was the same as that reported for the trans isomer.[13] The $[PdCl_2en]$ was prepared according to McCormick et al.[14] Anal. Calcd for $C_2H_8N_2Cl_2Pd$: C, 10.1; H, 2.99; N, 11.8; Cl, 29.9; Pd, 44.8. Found: C, 9.90; H, 2.83; N, 11.5; Cl, 29.9; Pd, 44.8. The *cis*-$[PtCl_2(NH_3)_2]$ was a gift from Matthey-Bishop, Inc., Malvern, Pa. Synthesis of *trans*-$[PtCl_2(NH_3)_2]$ was by the method of Kauffman and Cowan.[15] Anal. Calcd for $N_2H_6Cl_2Pt$: N, 9.34; H, 2.02; Cl, 23.6; Pt, 65.0. Found: N, 9.43; H, 2.14; Cl, 23.4; Pt, 65.1. The purity of the cis and trans isomers was checked by comparing the Raman spectra with literature spectra[16] and authetic samples. Synthesis of the rhodium(II) acetate monohydrate dimer was by the procedure of Legzdins et al.[17] Anal. Calcd for $C_8H_{16}O_{10}Rh_2$: C, 20.1; H, 3.37. Found: C, 20.3; H, 3.60. The $[Co(NH_3)_5OH_2](ClO_4)_3$ was prepared as described by Splinter et al.[18] Anal. Calcd for $N_5H_{17}O_{13}Cl_3Co$: N, 15.2; H, 3.72; Cl, 23.1; Co, 12.8. Found: N, 15.0; H, 3.50; Cl, 23.0; Co, 12.6.

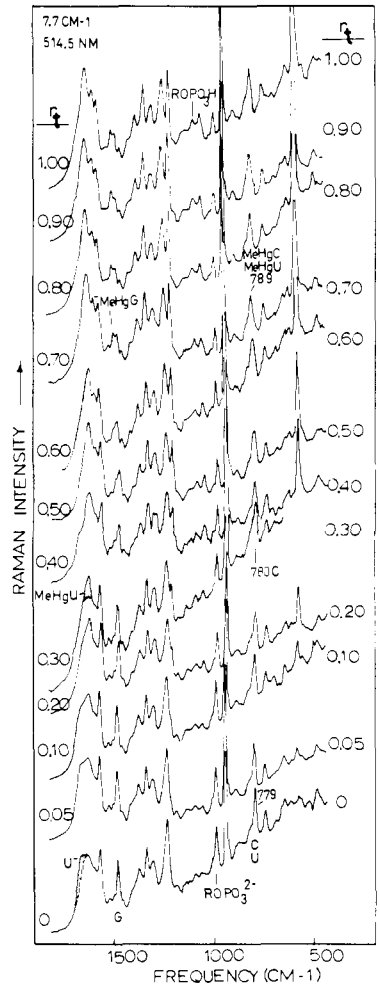

Figure 1. Raman spectra of solutions of $CH_3Hg(ClO_4)$ plus the four mononucleotide model as a function of r_t. Total $[ClO_4^-]$ = 100 mM, t = 25 °C, pH 7. Spex monochromator, scan conditions 10 s/step, 1-cm^{-1} steps. Slit widths and exciting frequency are indicated in the figure.

Model Nucleotide System. The model system solutions were prepared from stock solutions of the four nucleotides. These were obtained as follows: 5′-AMP, 5′-UMP, Aldrich Chemical Co., Milwaukee, Wis.; 5′-GMP, PL Biochemicals, Inc., Milwaukee, Wis., and Sigma Chemical Co., St. Louis, Mo.; 5′-CMP, Cyclo Chemical, Los Angeles, Calif., and Sigma. Some lots of commercial mononucleotides contain fluorescent impurities. The materials used here were selected for low background.

The solutions contained 50 or 100 mM ClO_4^- as an internal frequency and intensity reference. Solutions containing the metal complexes were prepared either by adding a weighed amount of the solid compound to give the desired r_t value (r_t = metal:total phosphate = metal:total base) to a mixture of the four nucleotides with 20 mM GMP, 20 mM CMP, 30 mM AMP, and 30 mM UMP, total phosphate 100 mM, or solutions of the nucleotides and of a slightly acidic solution of the metal complex were mixed to give the desired r_t value and diluted to give 100 mM total phosphate. The pH was adjusted, if necessary, to 7, using Radiometer PHM-4 or 64 pH meters. The solutions were passed through ultrafilters to remove particulate matter.

Raman Spectra. The general procedure for obtaining the Raman and Raman difference spectra (RADS) has been described previously.[10,19,20] Two different difference spectrometers were used, and both were controlled on-line by the same minicomputer system.[21] The first is built around a Spex 1400 monochromator and employs a Coherent Radiation

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(15) G. B. Kauffman and D. O. Cowan, *Inorg. Synth.*, **7**, 239 (1963).

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(21) J. C. English, R. W. Chrisman, and R. S. Tobias, *Appl. Spectrosc.*, **30**, 168 (1976).

CR-52G Ar^+ laser.[21,22] The second uses a Jobin-Yvon Ramanor HG-2 with a CR-8 Ar^+ laser, in some cases, pumping a CR-490 dye laser.[23] Spectra were processed off-line by using Program RAMAN,[10] and they were subjected to 17 or 25 point quartic moving point smoothing.

Data and Results

The Nucleotide Model. Raman Spectra of Mixtures of GMP, CMP, AMP, and UMP. The spectrum of the model is illustrated in Figure 1, $r_t = 0$. The base composition of the model approximates that of calf thymus DNA with UMP substituted for dTMP. In general, UMP and dTMP bind metals in an identical fashion. While dTMP was used in the first study of this type,[19] it was found that UMP has several advantages. The fluorescent background is lower, and its perturbation difference spectrum upon metalation is simpler.

The spectrum of the model is an almost exact superposition of the spectra of the individual nucleotides. This was verified by an examination of spectra obtained with solutions containing mixtures with [GMP] = [CMP] and [AMP] = [UMP] which had varying ratios of these two sets of nucleotides. The 11 spectra are illustrated in a figure in the microfilm edition, and the contributions to the principal bands are indicated. The two purines, GMP and AMP, have a number of vibrations at approximately the same frequencies as do the two pyrimidines, CMP and UMP, but these do not overlap. Consequently it will be easy to distinquish reactions of the purines from the pyrimidines but more difficult to distinguish between reactions of the two purines or of the two pyrimidines. Fortunately, there are enough unique bands or at least bands where the intensities of the two components differ so greatly that the contributions of the individual nucleotides and the band perturbations upon reaction usually can be measured.

Reaction with CH_3Hg^{II} in H_2O and D_2O at pH ~7, 25°C, $0 \leq r \leq 1$. The reaction of CH_3Hg^{II} as the perchlorate with a mixture of GMP, CMP, AMP, and dTMP at pH 7 has been studied previously for r_t ca. 0.2.[19] Twelve spectra of the GMP + CMP + AMP + UMP model in H_2O with CH_3Hg^{II} to give $0 \leq r_t \leq 1$ are illustrated in Figure 1. Reaction of CH_3Hg^{II} is quantitative throughout, since none of the characteristic frequencies of CH_3HgOH is visible. Particulary diagnostic of reaction is the absence of the Hg–O stretching band at 505 cm^{-1}. It is considerably easier to follow the reactions with the use of the difference spectra, model + CH_3Hg^{II} with varying r_t vs. model; these are illustrated in Figure 2.

To provide a data base for interpretation of the difference spectra, it is convenient to have fingerprint spectra for reaction of CH_3Hg^{II} with the individual nucleosides or nucleotides. These perturbation difference spectra for solutions with equimolar metal and nucleotide are illustrated in Figure 3. The evidence for the assignment of the binding site on a given nucleotide has been discussed previously.[8,10,24] From a comparison of Figures 2 and 3, it is seen that the principal reaction when $0 < r_t \leq 0.3$ is attack by CH_3Hg^{II} on uridine. At $r_t = 0.4$, weak features of the fingerprint for reaction with guanosine are visible, and these are very clearly defined by $r_t = 0.7$. A difference spectrum computed from the $r_t = 0.7$ minus the $r_t = 0.3$ spectrum of Figure 1 was almost the fingerprint for reaction at N(1) of GMP, indicating that reaction with GMP is the main process over this r_t range. An $r_t = 1.0$ minus r_t 0.7 difference was almost featureless except for weak bands consistent with reaction of CMP.

A quantitative description of the binding reactions can be obtained from plots of band intensity as a function of r_t as illustrated in Figure 4. The intensity of the $ROPO_3^{2-}$ stretch at 976 cm^{-1} was determined by casting a base line, using the minima on either side of the band and subtracting this from the maximum at 976 cm^{-1}. The integrated counts—of the order of 40K—were normalized for the different spectra by division by the perchlorate ν_1 intensity determined in an analogous fashion, ca. 130K counts.

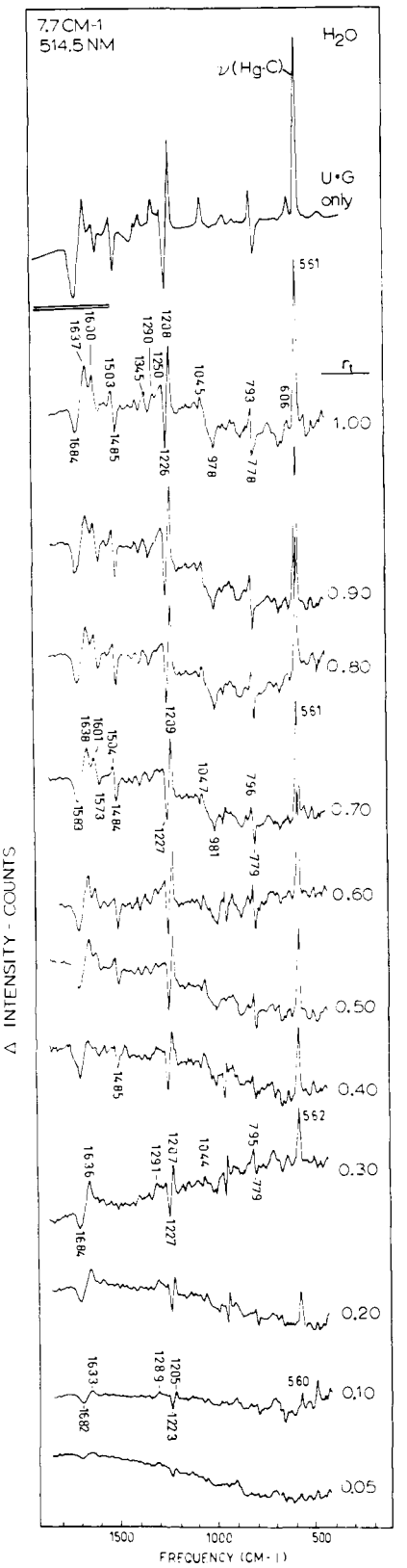

Figure 2. Raman difference spectra (RADS) for CH_3Hg^{II} plus the four-nucleotide model vs. the model as a function of r_t.

The intensity of the $ROPO_3^{2-}$ stretch decreases, because CH_3Hg^+ displaces protons upon reaction with UMP and GMP and these are transferred to the phosphate. This can be used to measure the extent of proton transfer. The decrease in intensity by $r_t = 0.8$ corresponds to liberation of 45 mM H^+, within the experimental error of the value computed for complete reaction with UMP and GMP, 50 mM. This also corresponds to an ca. 0.5 drop in solution pH.

(22) J. W. Amy, R. W. Chrisman, J. W. Lundeen, T. Y. Ridley, J. C. Sprowles, and R. S. Tobias, *Appl. Spectrosc.*, **28**, 262 (1974).
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(24) S. Mansy and R. S. Tobias, *J. Am. Chem. Soc.*, **96**, 6874 (1974).

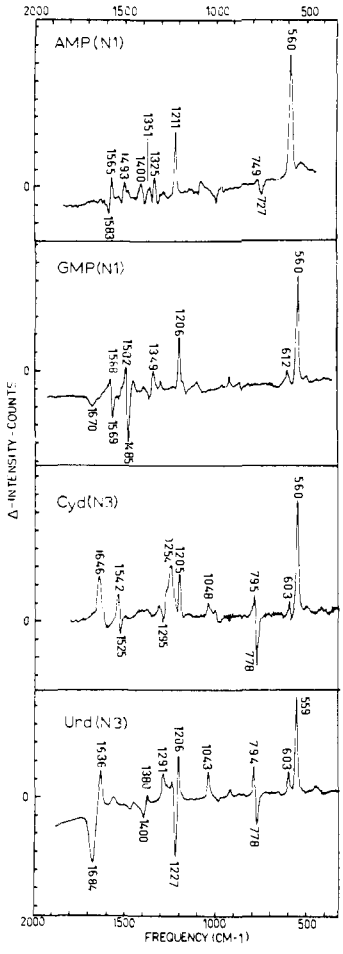

Figure 3. Raman perturbation difference spectra for methylmercuriation of individual nucleosides at pH 7, 25 °C. The spectra for uridine and cytidine are from ref 10; that for GMP is from ref 24. The concentrations of H_3CHg^{II} are equal and 50 mM. Scan conditions as in Figure 1.

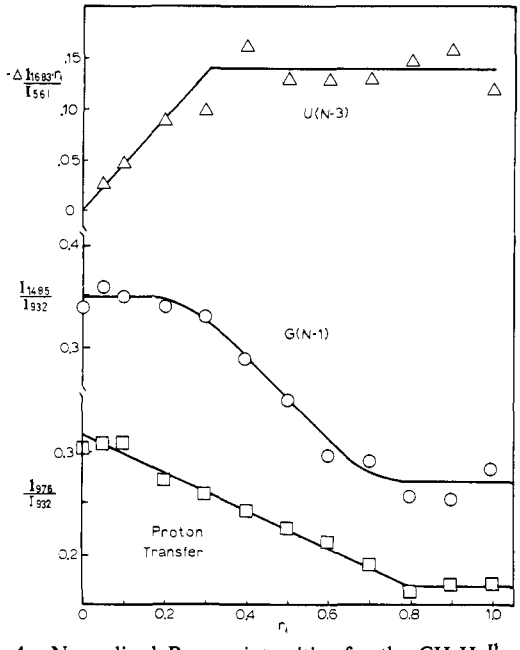

Figure 4. Normalized Raman intensities for the CH_3Hg^{II} reaction monitoring proton transfer from nucleotide base to phosphate, I_{976}/I_{932}; mercuriation at N(1) of GMP, I_{1485}/I_{932}; and mercuriation at N(3) of UMP, $-\Delta I_{1683}r_t/\Delta I_{561}$.

The intensity of the 1485-cm^{-1} GMP band was determined from the raw digital data in an analogous way and normalized, and

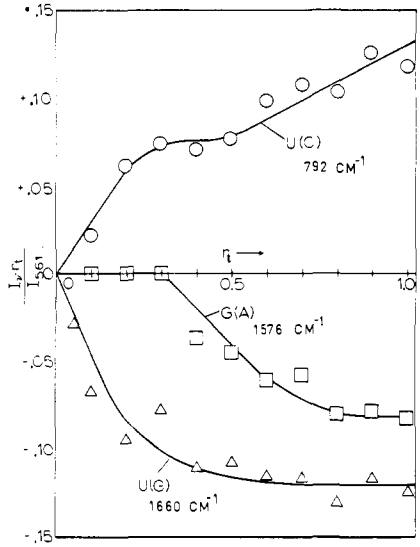

Figure 5. Normalized intensities for the CH_3Hg^{II} reaction in D_2O from the difference spectra: Δ, negative feature at ca. 1660 cm^{-1} caused by the decrease of scattering due mainly to UMP; □, negative feature at 1576 cm^{-1} caused by a decrease in GMP scattering; ○, positive feature due to mercuriation at N(3) of UMP and CMP.

these data also are plotted in Figure 4. These show that reaction with GMP at N(1) occurs over the range $0.3 < r_t < 0.8$. The best band for monitoring the UMP reaction is the one at 1680 cm^{-1} which decreases to ca. 1640 cm^{-1} upon metalation. Unfortunately it is obscured by water scattering in these dilute solutions. To follow it, the intensity of the negative feature in the difference spectrum, Figure 2, at 1684 cm^{-1} was determined. For convenience these were normalized by division with $\Delta I_{561}/r_t$, the normalized ν(Hg–C) intensities. These data also are plotted in Figure 4, and they indicate that mercuriation at N(3) of UMP is complete at r_t 0.3–0.4.

The evidence for CMP binding is less obvious, because this only takes place after reaction with UMP is complete and attack on GMP is occurring. It can be followed by a plot of the frequency at maximum intensity of the band in the 779–789 cm^{-1} region due to CMP and UMP. In the model, this occurs at 779 cm^{-1}. Mercuriation of UMP shifts its frequency to ca. 791 cm^{-1},[10] and this gives rise to a high-frequency shoulder on this band at r_t = 0.3 where reaction with UMP is essentially complete. Because CMP contributes most to the intensity of this envelope, the frequency at maximum intensity is unchanged. See Figure 1. Mercuriation of CMP increases the frequency to 788 cm^{-1},[10] and with $0.3 < r_t < 0.8$ the band maximum shifts to 789 cm^{-1} as the bulk of the CMP is mercuriated.

No evidence could be found for mercuriation of the base of AMP under these conditions. Figure 2 also illustrates a sum of the fingerprint spectra for mercuriation at N(3) of UMP and N(1) of GMP, scaled appropriately, and this is almost the same as the difference spectra for $r_t > 0.7$, confirming the nature of the principal binding reactions at lower r_t.

In the study of reactions in aqueous solution, it has been customary to collect vibrational data with D_2O and H_2O to obtain a complete data set. While it is easy to follow the decrease in UMP scattering at 1660 and 1700 cm^{-1} in the parent spectra because solvent scattering is absent from this region, it was found that the difference technique renders the use of both H_2O and D_2O solutions essentially superfluous. Eleven spectra for D_2O, $0.05 \leq r_t \leq 1.0$, are illustrated together with the corresponding difference spectra in the microfilm edition. A set of fingerprint spectra for mercuriation in D_2O is also given. These are not identical with the fingerprints in Figure 3 because of isotopic substitution on the nucleotides. In these solutions, the pD was controlled at 7, so no phosphate protonation occurred.

Almost all of the features in the difference spectra for $r_t \leq 0.6$ can be assigned to the fingerprints for reaction with UMP[10] or GMP[24] in D_2O. Intensities from the difference spectra are il-

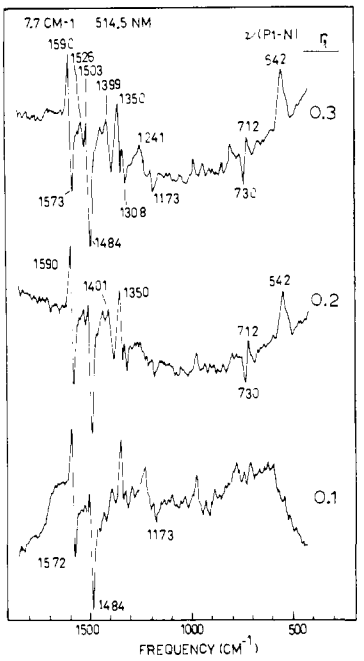

Figure 6. RADS for *cis*-$[PtCl_2(NH_3)_2]$ plus the four-monoucleotide model system as a function of r_t. Total $[ClO_4^-]$ = 50 mm, t = 25 °C. Scan conditons as in Figure 1.

lustrated in Figure 5. The decrease at ca. 1660 cm^{-1} is due mainly to mercuriation of UMP at N(3) with some contribution from mercuriation of GMP at N(1). The scattering at 1576 cm^{-1} is due to GMP and AMP, and the decrease is due to reaction of GMP. The scattering at 792 cm^{-1} is due to UMP and CMP when each is mercuriated at N(3). The initial increase in intensity is consistent with the decrease at 1660 cm^{-1}, and indicates mercuriation of UMP. The increase for $r_t \geq 0.6$ is consistent with mercuriation of CMP. The product distribution is essentially the same as that deduced for the H_2O solutions.

Reaction with *cis*-$[PtCl_2(NH_3)_2]$ at pH 7, 25° C. For comparison with the reactions of the aquodiammineplatinum(II) cations studied previously with *cis*- and *trans*-$[Pt(OH_2)_2(NH_3)_2](ClO_4)_2$, $r_t \leq 0.2$,[20] difference spectra were obtained for the chloro complexes, $0 < r_t \leq 0.3$. The difference spectra for *cis*-$[PtCl_2(NH_3)_2]$ are illustrated in Figure 6. The spectra are distinctly different from those of the CH_3Hg^{II} system.

A set of fingerprint spectra analogous to those in Figure 3 for platination of GMP, CMP, AMP, and UMP has been published previously.[20] The assignment of the binding sites also has been discussed in detail.[25,26] These spectra provide a data base for interpretation of Figure 6. The principal features all are due to reaction at N(7) of GMP *without proton loss* as may be seen from comparison of the figerprint spectrum for platination of GMP at N(7)[20,26] and the spectra in Figure 6. As was the case with *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, the small derivative feature with a minimum at 730 cm^{-1}, maximum at 710 cm^{-1}, plus the relatively low positive intensity at 1506 cm^{-1} are indicative of reaction with AMP.[20] The same product distribution is obtained regardless of whether the starting material is the chloro or aquo complex. In accord with this, the products of the reactions with these two complexes give the same ν(Pt–N) envelope with ν_S at 542 and a shoulder at ca. 520 cm^{-1}.

Reaction with *trans*-$[PtCl_2(NH_3)_2]$ at pH 7, 25 °C. For comparison with *cis*-$[PtCl_2(NH_3)_2]$, difference spectra were obtained for $0 < r_t \leq 0.3$; see Figure 7. The same data base used to interpret the difference spectra of the cis isomer can be used here, because it has been shown that the perturbations of the Raman spectra caused by binding at a particular site on a nucleotide are

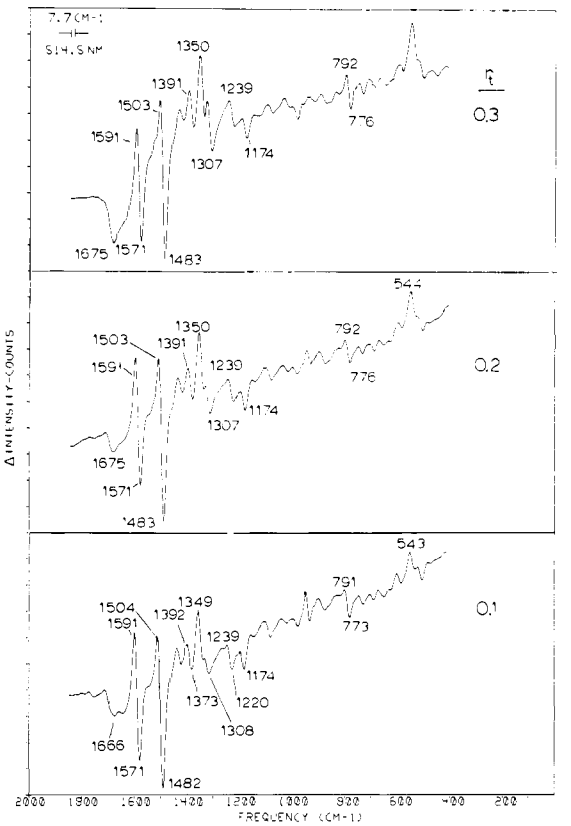

Figure 7. RADS for *trans*-$[PtCl_2(NH_3)_2]$ plus the four-mononucleotide model system as a function of r_t. Total $[ClO_4^-]$ = 50 m*m*, t = 25 °C. Scan conditions as in Figure 1.

essentially identical for the cis and trans isomers.[26] The difference spectra in Figure 7 are almost identical with the fingerprint for platination at N(7) of GMP.[27] In particular the trans isomer shows no measureable interaction with AMP (no derivative at 730 min, 710 cm^{-1} max) in contrast to the behavior of the cis isomer. This certainly is a real effect, because the same difference was observed with the aquo cations.[20] The ν(Pt–N) band appears at ca. 545 cm^{-1}, the same value found when *trans*-$[(H_3N)_2Pt(OH_2)_2]$[25] was the reactant.

The spectra for the maximum r_t value, 0.3, show weak features (777 min, 793 cm^{-1} max) consistent with slight attack on a pyrimidine. The minimum at 1675 cm^{-1} indicates involvement of UMP, but the features are not characteristic of N(3) binding and the phosphate $\nu_s(PO_3)$ indicates that there has been no proton transfer. This specrtrum also is distorted somewhat above 1400 cm^{-1} because the model fluoresced weakly, and the platinum quenched this.

Reaction of $[PdCl_2en]$ at pH 7, 25 °C. To study a palladium(II) compound with the cis structure, the ethylenediamine complex rather than the cis diammine was used, since the latter isomerizes rather rapidly to the trans form. The parent spectra $0 \leq r_t \leq 0.3$ are illustrated in the microfilm edition and the corresponding difference spectra are given in Figure 8.

While no set of fingerprint spectra for palladation of nucleotides analogous to those for mercuriation and platination is available, this is not necessary for an interpretation of Figure 8. As has been pointed out previously, platinum(II) and mercury(II) binding produce almost identical perturbation difference spectra so long as coordination *occurs at the same site*.[25] Similar effects even occur with electrophiles as diverse as CH_3^+ or $H^+(D^+)$,[8] so the effect of binding of $enPd^{II}$ at a particular site on the Raman spectrum can be predicted rather accurately.

The $enPd^{II}$ reaction shows little selectivity for pyrimidine vs. purine nucleotides, and even the r_t = 0.025 spectrum shows features characteristic of reaction with both kinds of nucleotide. The

(25) G. Y. H. Chu, R. E. Duncan, and R. S. Tobias, *Inorg. Chem.*, **16**, 2625 (1977).
(26) G. Y. H. Chu, S. Mansy, R. E. Duncan, and R. S. Tobias, *J. Am. Chem. Soc.*, **100**, 593 (1978).
(27) See Figure 3 of ref 20.

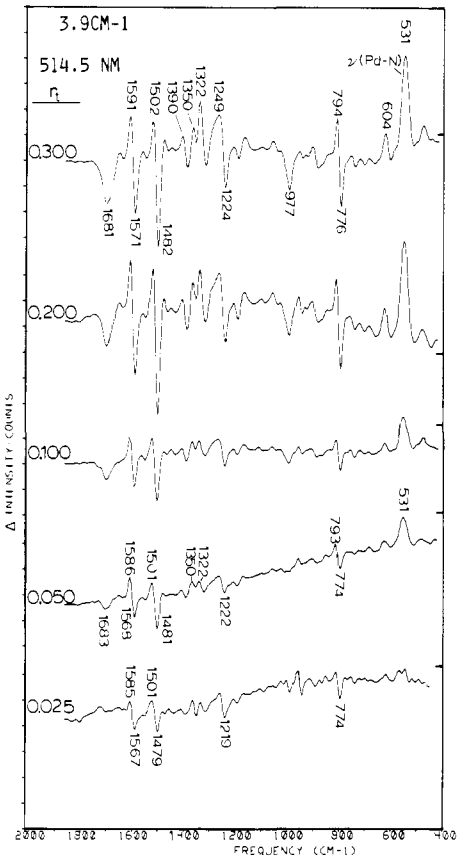

Figure 8. RADS for $[PdCl_2en]$ plus the four-nucleotide model vs. the model as a function of r_t. Total $[ClO_4^-]$ = 50 mM. Scan conditons as in Figure 1.

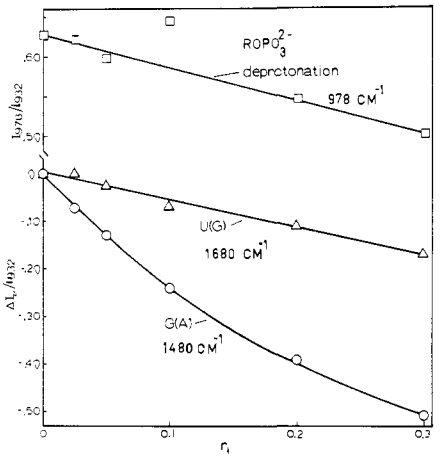

Figure 9. Normalized Raman intensities for the reactions of $enPd^{II}$: Δ, negative feature in difference spectrum at ca. 1680 cm^{-1}; O, negative feature in difference spectrum at ca. 1480 cm^{-1}; □, phosphate stretching intensity.

spectra with $r_t \leq 0.1$ are chracterized by features due to reaction at N(7) of GMP and N(3) of UMP with substitution of the metal for a proton. At higher r_t values, there also are features characteristic of reaction with AMP and CMP; cf. the fingerprint spectra for reaction of the platinum(II) complexes. These reactions have little effect on the vibrations of the $enPd^{II}$ moiety. The most intense (Pd–N) stretching mode is observed at 534 cm^{-1} [28] with crystalline $[PdCl_2en]$, while the analogous vibration in the nucleotide system is at 531 cm^{-1}.

Normalized intensities from the difference spectra are illustrated in Figure 9. The decrease in scattering at ca. 1680 cm^{-1} results mainly from attack at N(3) of UMP, while the decrease at ca.

(28) R. W. Berg, *Spectrochim. Acta, Part A*, **31**, 1409 (1975).

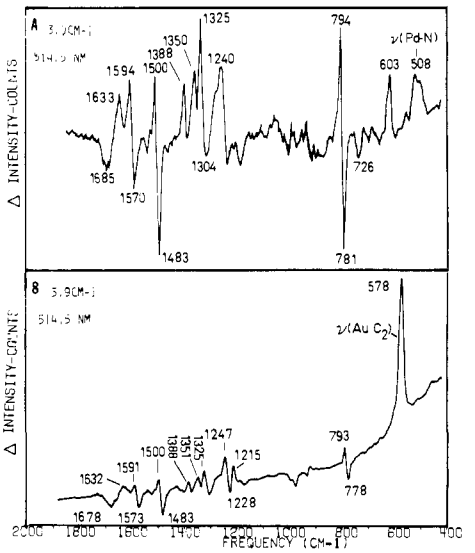

Figure 10. (A) RADS for *trans*-$[PdCl_2(NH_3)_2]$ plus the four-nucleotide model vs. the model in H_2O, r_t = 0.2, $[ClO_4]$ = 50 mM, t = 25 °C. Jobin-Yvon monochromator. (B) RADS for $(CH_3)_2Au(ClO_4)$ plus the four-nucleotide model vs. the model, $[ClO_4]$ = 100 mM, t = 25 °C. Spex monochromator. Scan conditions as in Figure 1.

1480 cm^{-1} is due mainly to reaction of GMP. The pH of the system dropped slightly upon reaction with $[PdCl_2en]$, and the decrease in phosphate scattering at ca. 978 cm^{-1} indicates release of only ca. 22 mM H^+ at r_t = 0.3. This means that, while attack at N(3) of UMP displaces a proton, $enPd^{II}$ binds to GMP at N(7) without extensive deprotonation as also is observed with *cis*-$(H_3N)_2Pt^{II}$, vide supra.

To check for completeness of reaction, a spectrum was obtained for a solution of $[PdCl_2en]$ + the four nucleotide model, r_t = 0.2, that had been allowed to equilibrate for 2 weeks. To minimize the photodecomposition of the palladium(II) complex, a dye laser was used to excite the spectrum at 594 nm. No difference from the spectrum discussed above could be detected. A similar product distribution was determined from a spectrum for r_t = 0.2 obtained with a D_2O solution.

Reaction of *trans*-$[PdCl_2(NH_3)_2]$ at 7, 25 °C. The set of spectra for r_t = 0.2 is illustrated in Figure 10A. The difference spectrum is essentially identical with that for $[PdCl_2en]$. Some minor differences are due to the higher resolution of the monochromator used in recording the spectra with the trans isomer. These data indicate that the distribution of palladium in the complexes with different nucleotides is similar with *cis*-$enPd^{II}$ and *trans*-$(H_3N)_2Pd^{II}$. There is one significant difference between the two palladium complexes. Although the samples had been allowed to react for 2–3 days before the spectra were determined, the extent of reaction is considerably greater for the trans complex. For example, the parent spectra available in the microfilm edition indicate a comparable extent of reaction for *trans*-$(H_3N)_2Pd^{II}$, r_t = 0.2, and $enPd^{II}$, r_t = 0.3. Spectra also were recorded with D_2O, r_t = 0.2, and the same effect was observed there.

Reactions with $(CH_3)_2Au^{III}$ at pH 7, 25 °C. A spectrum for the system with $(CH_3)_2Au^{III}$ as the perchlorate, r_t = 0.2, is illustrated in Figure 10B. Reaction of $(CH_3)_2Au^{III}$ is quantitative, since the solution is homogeneous, and the solubility of $(CH_3)_2AuOH$ in water is only ca. 2.5 mM.[29] The spectrum clearly shows features at 578 and 1215 cm^{-1} characteristic of the $(CH_3)_2Au^{III}$ moiety.[30] The frequencies are similar to those of the complex $[(CH_3)_2Au(NH_3)_2]^+$, $\nu_s(Au-C_2)$ 579, $\delta_s(CH_3)$ 1220 and 1249 cm^{-1}.[31] The very small separation between $\nu_s(Au-C_2)$ and $\nu_{as}(Au-C_2)$—the bands are unresolved—indicates that

(29) S. J. Harris and R. S. Tobias, *Inorg. Chem.*, **8**, 2259 (1969).
(30) M. G. Miles, G. E. Glass, and R. S. Tobias, *J. Am. Chem. Soc.*, **88**, 5738 (1966).
(31) H. Hagnauer, G. C. Stocco, and R. S. Tobias, *J. Organomet. Chem.* **46**, 179 (1972).

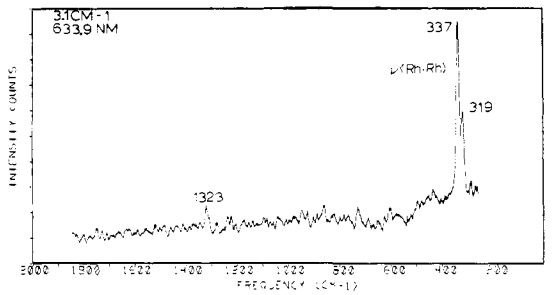

Figure 11. RADS for $Rh_2(OOCCH_3)_4$ plus the four-nucleotide mixture, $r = 0.12$, total $[ClO_4^-] = 50$ mM, $t = 25$ °C. Jobin-Yvon monochromator, spinning sample cell.

$(CH_3)_2Au^{III}$ is behaving as a bifunctional electrophile toward the nucleotides. Coordination of dissimilar ligands usually leads to a significant separation of ν_s and ν_{as}.

The perturbations caused by coordination of $(CH_3)_2Au^{III}$ and CH_3Hg^{II} to pyridine are almost identical,[31] so as predicted it should be possible to use the perturbation difference fingerprints in Figure 3 to interpret Figure 10B. The $(CH_3)_2Au^{III}$, $r_t = 0.2$, difference spectrum is rather like the CH_3Hg^{II} spectra with $r_t \geq 0.7$. The negative features at 1573 and 1483 cm^{-1} arise from purine binding, while the derivative with maximum at 793 and minimum at 778 cm^{-1} is characteristic of pyrimidine binding. The negative feature at 1978 cm^{-1} indicates attack at the N(3) and N(1) nitrogens of UMP and GMP, respecitvely. The features from 1247 to 1388 cm^{-1} indicate reaction with CMP and AMP. Clearly the spectra indicate that there is no particular selectivity in the coordination of $(CH_3)_2Au^{III}$ to pyrimidine over purine nucleotides.

From a comparison of the difference spectra in Figure 10 or between Figures 8 and 10, it can be seen that the bands are very similar for the $r = 0.2$ $(CH_3)_2Au^{III}$, $enPd^{II}$, and *trans*-$(H_3N)_2Pd^{II}$ systems once the bands due to internal vibrations of $(CH_3)_2Au^{III}$, 577 and 1215, $enPd^{II}$, 531, and *trans*-$(H_3N)_2Pd^{II}$, 508 cm^{-1}, are deleted.

Reaction with $[Rh(O_2CCH_3)_2 \cdot H_2O]_2$, $r_t = 0.12$, pH 7. This green rhodium(II) complex is only very slightly soluble in water, but it dissolves readily in the four-nucleotide model showing that reaction occurs. The absorption spectrum of a solution with $r_t = 0.14$ was obtained by diluting to a total phosphate concentration of 0.02 M. This red solution has ϵ_{max} at 548 nm. Because of the strong absorption of the complex except in the red, excitation of the Raman spectrum was at 633.9 nm at which wavelength the absorbance was ca. 0.7. A spinning cell was employed. In order to compute a difference spectrum, it was necessary to scale the reference, using the intensity of ν_1 of the internal ClO_4^- reference. The difference spectrum shows only two intense bands at 337 and 319 cm^{-1} (relative intensities 1 and 0.5). This is illustrated in Figure 11. These frequencies are characteristic of the $RH_2(OOCCH_3)_4$ cluster. For example, we measured bands at 351 and 332 cm^{-1} (relative intensities 1 and 0.5) for a tetrahydrofuran solution of $Rh_2(OOCCH_3)_4$ and microcrystalline $Rh_2(OOCCH_3)_4 \cdot 2H_2O$ was reported to have a band at 320 cm^{-1}.[32]

The only other feature above the noise level in the difference spectrum is a positive feature at 1323 cm^{-1} apparently resulting from a weak perturbation of the AMP scattering at 1304 cm^{-1}.

Reaction with $[Co(NH_3)_5OH_2]^{3+}$, $r_t = 0.2$, pH 7. Addition of a solution of $[Co(NH_3)_5OH_2](ClO_4)_3$ to the four-mononucleotide model causes an immediate change in the adsorption spectrum from that of $[Co(NH_3)_5OH_2]^{3+}$ to that of a mixture of the aquo cation and $[Co(NH_3)_5OH]^{2+}$, since the p^*K_1 value of the aquo complex is 6.2.[18] At pH 7.2, the solution has λ_{max} values of 349 and 500 nm. These are rather similar to those reported for a product suggested to be $[Co(NH_3)_5ADP]$, 353 and 513 nm.[33] In neither case do the spectra correspond to that reported by Schmidt and Taube[34] for $[Co(NH_3)_5PO_3OH]^+$, suggesting that there is incomplete inner-sphere coordination of the phosphate.

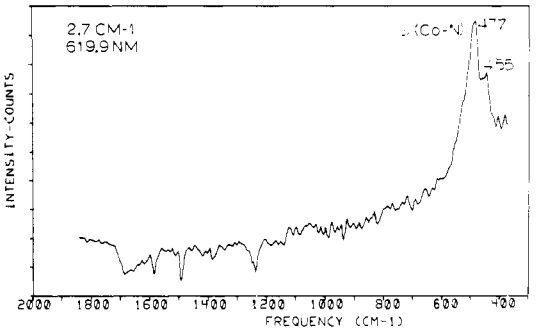

Figure 12. RADS for $[Co(NH_3)_5OH_2](ClO_4)_3$ plus the four-nucleotide mixture, $r_t = 0.2$, total $[ClO_4^-] = 100$ mM, $t = 25$ °C. Scan conditions: 5 s/step, 1-cm^{-1} step.

Because of the absorption of this system at shorter wavelengths, the Raman spectrum was excited at 619.6 nm. The reference was scaled, using the internal ClO_4^- ν_1. The only indisputably real feature in the difference spectrum illustrated in Figure 12 is the band at 477 cm^{-1} with a shoulder at ca. 455 cm^{-1}. This is due to (Co–N) stretching; cf. $[Co(NH_3)_5SO_4]Cl$, 490,[35] $[Co(NH_3)_5Cl]Cl_2$, 485 vs, 463 m, 441 m cm^{-1} [36] from solid-state spectra. Although the features in the spectrum cancel within the noise level below 1200 cm^{-1}, there is a slight mismatch at higher frequencies, apparently because the long period of irradiation of the sample caused slight decomposition at the cell windows. It should be noted that the ordinate in Figure 12 is expanded more than in the other spectra to magnify any differences.

Reaction of 0.3 M $[Co(NH_3)_5OH_2](ClO_4)_3$ with 0.1 M GMP gave a rose-colored precipitate immediately. The analysis corresponded approximately to $[Co(NH_3)_5]_2(GMP)_3 \cdot 8H_2O$.[37] In an analogous reaction with 0.3 M AMP, a rose precipitate was obtained after standing for several hours at room temperature. The analysis corresponded approximately to $[Co(NH_3)_5](AMP)(ClO_4) \cdot 3H_2O$.[37] The phosphate vibrations were essentially unperturbed as determined from IR spectra of the compounds: GMP complex, 970; GMP, 976; AMP complex 976; AMP, 974 cm^{-1}.

Discussion

Determination of Product Distributions in Labile Systems with Competitive Reactions by Raman Perturbation Difference Spectroscopy. Although we have used Raman difference spectroscopy (RADS) in two limited studies of competitive reactions,[19,20] this is the first detailed application of this technique. The competitive reaction technique is a relatively common one for inert systems where the product distribution can be determined by, for example, chromatography. For labile systems, a spectroscopic technique that can establish the product distribution in situ seems called for.

UV absorption spectrophotometry lacks the capbility of resolving products from reactions of different nucleotides and gives little information on binding sites, and IR studies are hampered by the strong water absorption. Because the sensitivity of RADS permits the study of millimolar concentrations of nucleotides under nonresonant conditions[21] and much lower concentrations with a tunable UV laser,[38] we have used this technique.

A large number of data were collected for the reactions of CH_3Hg^{II} to test the accuracy of this new technique. A comparison

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(33) R. D. Cornelius, P. A. Hart, and W. W. Cleland, *Inorg. Chem.*, **16**, 2799 (1977).
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(35) R. Coomber and W. P. Griffith, *J. Chem. Soc. A*, 1128 (1968).
(36) T. M. Loehr, J. Zinich, and T. V. Long, *Chem. Phys. Lett.*, **7**, 183 (1970).
(37) For $[Co(NH_3)_5](GMP)_3 \cdot 8H_2O$. Anal. Calcd for $C_{30}H_{82}N_{25}O_{32}P_3Co_2$: C, 23.8; H, 5.45; N, 23.1. Found: C, 25.3; H, 5.55; N, 23.3. For $[Co(NH_3)_5](AMP)(ClO_4) \cdot 3H_2O$. Anal. Calcd for $C_{10}H_{33}N_{10}ClO_{14}PCo$: C, 18.7; H, 5.17; N, 21.8. Found: C, 19.1; H, 4.95; N, 21.8.
(38) T. H. Bushaw, F. E. Lytle, and R. S. Tobias, *Appl. Spectrosc.*, **32**, 585 (1978).

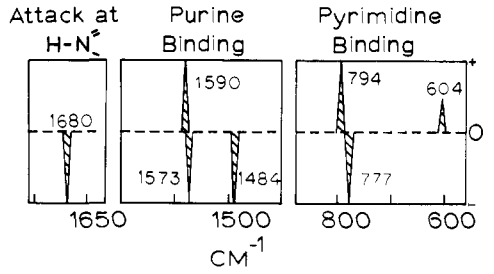


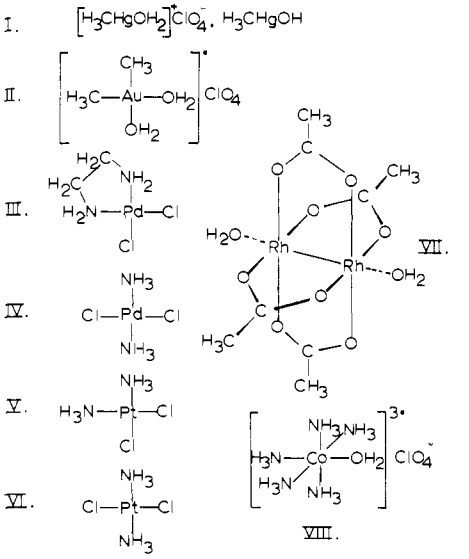
Figure 13. Marker bands in Raman difference spectra for reactions of heavy metals with nucleotides.

of the parent and difference spectra, e.g., in Figures 1 and 2, quickly demonstrates the utility of the difference technique in studying small amounts of reaction. Although a detailed interpretation of the Raman and Raman difference spectra for these systems can be rather tedious, there are a few simple spectral features that permit one to deduce at a glance a good deal about the reactions occurring. These are summarized in Figure 13.

Binding at N(3) to either pyrimidine nucleotide, CMP or UMP, has been observed previously[8,10,25] in studies with individual nucleosides or nucleotides to give a very characteristic shift to higher frequency of the ligand band at 783–784 cm^{-1}. This results in a strong derivative signal in the difference spectrum. There is no interference in this region from complexation of either AMP or GMP. Similarly binding to either purine nucleotide[24,26] gives characteristic signals in the 1450–1600-cm^{-1} region, and there is no interference from either pyrimidine in this region. Deprotonation by metalation at N(3) of UMP or N(1) of GMP is signaled by a negative feature in the difference spectrum at ca. 1680 cm^{-1} due to the shift to lower frequency of modes involving primarily carbonyl stretching. Since these are the highest frequency modes, this effect is not obscured by any other reactions.

For a more detailed analysis of the reactions, recourse must be made to fingerprint spectra for metalation at a given site and intensity data. Figures showing the rather smooth trends in frequencies upon electrophilic attack by H_3CHg^+, $(H_3N)_2Pt^{2+}$, $H^+(D^+)$, and CH_3^+ on GMP (N(7)), AMP N(1), Ino N(1) and N(7), Urd N(3), and Cyd N(3) have been published recently.[8] The characteristic frequencies of the H_3CHg^+–nucleotide difference spectra have been tabulated previously.[24] For the determination of a product distribution, it is necessary to quantitate the reactions, using the Raman intensities. In some cases it was possible to select bands relatively free from interference and to determine their intensity. Envelope resolution, while practical with very simple spectra, is not practical with a system such as the nucleotide mixture. The difference intensities offer an advantage in that interference due to bands which are not perturbed by reaction is removed, although their photometric accuracy is lower.

Selectivity in Electrophilic Attack by Metal Complexes on Nucleic Acid Constituents. These studies were undertaken to determine if different metal complexes exhibit different selectivity in their reactions with nucleic acid constituents. Extensive studies on reaction specificity of alkalating agents with nucleosides have been made and are summarized by Singer.[39] The metal complexes chosen, I–VIII, include species such as CH_3Hg^{II} that has long been known to bind strongly to nucleic acid constituents[40,41] and which finds use as a denaturing agent.[42] The *cis*-diammineplatinum(II) complexes have been studied widely, because their antimitotic activity is believed to derive from electrophilic attack on nuclear DNA.[43] The trans isomer shows no useful activity, so any differences in cis and trans reactivity are of interest. Diamminepalladium(II) compounds have been studied by several groups, because they are isostructural with the platinum(II) complexes and might be expected to react similarly.[44-47] *cis*-



$(H_3C)_2Au^{III}$ is isoelectronic and isostructural with the cytotoxic *cis*-$(H_3N)_2Pt^{II}$. One of the few other transition metals to exhibit antitumor activity is rhodium, and the rhodium(II) carboxylates are, perhaps, the most effective.[48] The selectivity of cobalt(III) complexes has been attributed to interactions of exocyclic groups on the nucleic acid constituent with other ligands bound to cobalt.[4]

In terms of their selectivity, these metal complexes fall into four groups. (1) The CH_3HgClO_4 (or CH_3HgOH) is unique among these species in that it attacks specifically at the N–H bonds; i.e., it coordinates to N(3) of UMP, and when $r_t \geq 0.4$ and all the UMP has been used up, it binds to N(1) of GMP. (2) Three of the complexes, $[PdCl_2en]$, *trans*-$[PdCl_2(NH_3)_2]$, and $(CH_3)_2AuClO_4$, bind strongly to the nucleotides but show no special tendency to attack at N–H bonds in preference to ring nitrogens such as N(7) of GMP, N(3) of CMP, etc. (3) *cis*- and *trans*-$[PtCl_2(NH_3)_2]$ show quite different binding reactions from any of the complexes discussed above. First, there is little tendency for attack at N–H bonds with $r_t \leq 0.3$. Furthermore, the platinum complexes show high selectivity for purine over pyrimidine nucleotides. Even at $r_t = 0.3$, where the complex is equivalent to 60% of the total bases, reaction of the cis isomer is almost entirely with the GMP and AMP. The trans isomer shows high selectivity for GMP and little reaction with AMP even at $r_t = 0.3$. This surprising result was also noted in the reaction of *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, $0 < r_t \leq 0.2$, so there is little doubt that the two platinum isomers exhibit somewhat different selectivity.

The fourth group includes $[Rh_2(OOCCH_3)_4]$ and $[Co(NH_3)_5OH_2](ClO_4)_3$, which gave no significant perturbation of the nuclotide vibrations and, consequently, for which no deductions can be made concerning the nature of the reactions.

Product distributions in these systems are summarized in Table I for r_t values where the metal, if it reacts completely, is equivalent to 40% of the bases.

Factors Affecting Selectivity. First, it should be emphasized that pH will have a major effect on the product distribution in systems such as these. The distributions have been examined here only at pH 7, since the main interest in these processes is their effect on nucleic acids at physiological pH.

The product distribution for methylmercury(II) can be understood in the following way. Substitution reactions are very

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Table I. Product Distributions (Percent Reaction of Nucleotides) and Binding Sites in the Metal Complex–Four Nucleotides Model Systems at pH 7, 25 °C

		nucleotide binding site				
electrophile	r_t	GMP	CMP	AMP	UMP	H^+ released, mM
$H_3CHgClO_4$	0.4	30 N(1)	0	0	~100 N(3)	27
cis-$(H_3C)_2AuClO_4$	0.2	35	slight N(3)	slight	25 N(3)	
[$PdCl_2$en]	0.2	60 N(7)	moderate N(3)	moderate	50 N(3)	10
trans-[$PdCl_2(NH_3)_2$]	0.2	80 N(7)	moderate N(3)	moderate	65 N(3)	
cis-[$PtCl_2(NH_3)_2$]	0.2	100 N(7)	0	50	0	0
trans-[$PtCl_2(NH_3)_2$]	0.2	100 N(7)	~0	none	~0	0

fast,[49] the cation is unifunctional, and no hydrogen-bonding interactions should occur between the methyl group and the nucleotides. Consequently, selectivity must be thermodynamically controlled and based upon metal–ligand bond strength. Methylmercury(II), although a soft acid, has been observed to give good linear free energy realtions between protonation and complexation with nitrogen bases.[49] The selectivity observed here is the same as the proton basicity, $H_{-1}UMP^- > H_{-1}GMP^- > CMP > AMP$. Significant binding to AMP does not occur, because binding reactions which are not at least as favorable as that of $ROPO_3^{2-}$ will be blocked (total phosphate = 100 mM). Equilibrium constants for $CH_3HgOH + L \rightleftharpoons CH_3HgL + OH^-$ with L = cytidine N(3), adenosine N(1), and $ROPO_3^{2-}$ are estimated to be log K = −4.9,[50] −6.5,[50] −4.8 (value for $HOPO_3^{2-}$ [52]).

The selectivity of the diammineplatinum(II) complexes may be understood partially on the basis of their substitution kinetics. These are classically inert complexes, and ligand nucleophilicity will be the dominant factor.[53] No significant attack occurs at N(3) of UMP or N(1) of GMP since these are protonated at pH 7 and consequently have very low nucleophilicity. The basis for the high selectivity of the diammineplatinum(II) species for purine over pyrimidine nucleotides is less obvious. The proton basicity stands in the order CMP N(3) > AMP N(1) > GMP N(7), pKs ca. 4.3, 3.8, and 2.3, respectively.[54] Scovell and O'Connor[55] determined equilibrium constants for reactions of ca. 10^{-4} M *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ with cytidine, adenosine, and guanosine at pH 6.5, 25 °C, and found that the values were all about the same: log K_1 = 3.5, 3.6, and 3.7, respectively. Nevertheless, GMP is the best nucleophile. In other studies, it has been observed that platinium(II) substitutions show no correlation with proton basicity or the stability of CH_3Hg^{II} complexes.[56]

The square-planar complex [$PdCl_2$en] is substitutionally inert, but its reactions are about 10^5 faster than those with the analogous *cis*-[$PtCl_2(NH_3)_2$],[53] and Martin and co-workers[44] have reported that reactions of enPdCl_2 and nucleosides are resonably fast. It was also suggested[44a] that "*cis*-dichloro Pd(II) complexes may be better models for intracellular action of the corresponding Pt(II) complexes that the Pt(II) complexes themselves". It should be noted that, presumably because of their greater inertness, the analgous platinum(II) complexes give very different products when several nucleotides are allowed to complete for a limited amount of the complex (vide supra). With [$PdCl_2$en] there is attack at N–H bonds of UMP with displacement of a proton, analogous to the behavior of H_3CHg^{II}, but N(7) binding to GMP, analogous to the [$PtCl_2(NH_3)_2$] isomers, is indicated. In addition, reaction with CMP and AMP is indicated.

The change from cis to trans stereochemistry with palladium(II) has little effect on product distribution. The one significant difference is that, at r = 0.2, *trans*-[$PdCl_2(NH_3)_2$] shows ca. 50% more reaction than [$PdCl_2$en]. The cis isomer may be less reactive because of the stability of the binuclear hydroxo complex or because it forms a more stable complex with the phosphate.

The square-planar $[(CH_3)_2Au(OH_2)_2]^+$ species is isoelectronic and isostructural with $[(H_3N)_2Pt(OH_2)_2]^{2+}$, but substitutions at the gold center are very fast.[57] Consequently, the species distribution should be thermodynamically determined as with CH_3Hg^{II}. As was the case with the mercurial, there is attack at N–H bonds, and the reactivity pattern is similar to that of [PdenCl$_2$] and *trans*-[$PdCl_2(NH_3)_2$]. Where other comparisons are possible, the solution chemistry of $[enPd(OH_2)_2]^{2+}$ and $[(CH_3)_2Au(OH_2)_2]^+$ seems rather similar. Both form binuclear hydroxo complexes, reactions 1 and 2, and are slightly weaker aquo acids than $CH_3HgOH_2^+$.[58]

$$2[enPd(OH_2)_2]^{2+} \rightleftharpoons 2H_3O^+ + [enPd(OH)_2Pden]^{2+} \qquad \log {}^*\beta_{22} = -8.33^{58} \quad (1)$$

$$2[(CH_3)_2Au(OH_2)_2]^+ \rightleftharpoons 2H_3O^+ + [(CH_3)_2Au(OH)_2Au(CH_3)_2] \qquad \log {}^*\beta_{22} = -9.79^{29} \quad (2)$$

Tetra-μ-acetato-dirhodium(II) behaves quite differently in that it causes almost no measureable perturbation of the electron density of the nucleotides upon coordination. That coordination does occur is assured from the changes in the visible spectrum as $[Rh_2(OOCCH_3)_4]\cdot 2H_2O$ is reacted with the four-nucleotide mixture. A green color is characteristic of the solid compound or a solution in 0.1 M pH 7 phosphate buffer, while the solution of the nucleotide mixture is red. This is characteristic of $[Rh_2(OOCCH_3)_4]$ with two axially coordinated *nitrogen* donors.[59] The Raman scattering caracteristic of the $[Rh_2(OOCCH_3)_4]$ cluster shows that the Rh–Rh bond is not broken. Apparently, the involvement of the rhodium orbitals in the metal–metal bond minimizes the perturbation of the electron density of the nucleotides. The thermodynamic stability of the AMP complex, log K_1 = 3.28,[60] may result in part from interactions of the exocylic amino group of AMP with the carboxylate oxygens. An analogous hydrogen bonding has been noted by Sorrell et al. between deoxyadenosine and the oxygen of coordinated acetylacetonate.[61]

While the rhodium(II) carboxylates show antitumor activity in animal studies and[48,59,62] are one of the few transition-metal systems to do so aside from the *cis*-diammineplatinum(II) complexes, it is unlikely that interaction of the parent carboxylate is involved in the same way as electrophilic attack by a platinum(II) complex.

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The $[Co(NH_3)_5OH_2]^{3+}$ ion was chosen as an example of a substitutionally inert, unifunctional electrophile involving a first row transition metal ion. There is relatively little information on Co(III)-nucleotide complexes. Kistenmacher and Marzilli found that $[Co(acac)_2(NO_2)_2]^-$ reacted readily in aqueous solution with adenine but not cytosine, uracil, or guanine nucleotides to yield $[Co(acac)_2(NO_2)(dAdo)]$.[61] Coordination of deoxyadenosine was via N(7). With 25 mM *cis*-β-$[Co(trien)Cl_2]^+$ and ca. 10^{-4} M nucleosides, the following percent reaction values were obtained after 3 days in aqueous solution: dT, 65; dG, 50; dC, 10; dA, 0.[63] In the reaction between $[Co(NH_3)_5OH_2]^{3+}$ and the four-nucleotide mixture, outer-sphere complexing with the phosphate is suggested by the visible spectrum, the lack of measurable perturbation of the Raman-active vibrations, and the products precipitated from more concentrated GMP and AMP solutions. Cleland and co-workers[33] obtained a species by reaction of $[Co(NH_3)_5OH_2]^{3+}$ in solution which they felt was $Co(NH_3)_5ADP$, although it could not be isolated or characterized. Coordination via the phosphate was tacitly assumed, although there was no proof of this.

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Acknowledgments. We should like to thank Englehard Industries and Matthey Bishop, Inc., for loans of platinum. R. S. Tobias would like to thank the National Science Foundation for a NATO Senior Fellowship during the tenure of which a portion of this work was carried out. He also is indebted to Professor W. Beck of the University of Munich for Accommodations in his laboratory and many helpful discussions.

Supplementary Material Available: Raman spectra (6 pages). Ordering information is given on any current masthead page.

CIDNP-Detected Nuclear Resonance of Transient Radicals in Pulse Radiolysis

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Abstract: CIDNP detection of nuclear resonance in transient radicals produced by pulse radiolysis is described. CIDNP intensities of the reaction products can be perturbed by the application of radio frequency corresponding to the nuclear resonance in the transient radicals. In addition to the measurement of hyperfine coupling constants of transient radicals, the pulsed nature of the variable-field CIDNP nuclear resonance technique in pulse radiolysis can be used to measure radical kinetics and to study CIDEP population dynamics in various magnetic fields.

Introduction

The study of transient radical structure and dynamics in liquids has been a continuing challenge of magnetic resonance spectroscopy. While substantial improvements in the time-resolved EPR technique have been made,[1,2] ENDOR studies of transient radicals in solution have not yet been achieved. Nuclear resonance of transient radicals in liquids, however, can be detected via the CIDNP effect.[3] The CIDNP detected nuclear resonance experiment in variable magnetic fields is combined with the pulsed electron beam as a means of studying transient radicals.

The ease of observation of CIDNP in pulse radiolysis in both ^1H and natural abundance ^{13}C nuclei has been demonstrated.[4,5] In these experiments, the electron pulse irradiation is carried out in a variable magnetic field, and the reaction products are transferred to the NMR probe by using a fast flow system (Figure 1). The observation of CIDNP in various products of pulse radiolysis is possible because the transfer of the reaction products into the NMR probe is affected before appreciable nuclear spin relaxation takes place.

Here, we wish to illustrate further development of this experimental approach; where a radio frequency (rf) irradiation is supplied during the radical reaction in the variable magnetic field. When the appropriate radio frequency is applied during the radical lifetime, the electron nuclear spin populations of the transient radical are perturbed, resulting in a change of the CIDNP intensities in the radical reaction products.

The first demonstration of CIDNP-detected nuclear resonance was carried out by Sagdeev and co-workers.[3] In their experiments, radicals were produced by photolysis in the NMR probe. The continuous rf irradiation was carried out in the NMR probe itself. In the pulse radiolysis experiments to be discussed below, the pulsed nature of the excitation and the variable magnetic field of the reaction illustrates many new possibilities of the CIDNP-detected nuclear resonance in radicals.

The pulsed nature of the experiment allows the use of pulsed rf, thus high H_1 fields are available without the associated problems of sample heating. H_1 in the experiments to be described was in the 100-200-G range. Also, the pulsed nature of the experiment transforms what is an essentially steady-state CIDNP experiment into a time-resolved one, where time dependence, i.e., radical kinetics, of transient radicals can be studied. Furthermore, the rf power dependence of CIDNP-detected nuclear resonance (CIDNP-NR) allows differentiation of slower and faster radical pathways and thus CIDNP processes. The variable magnetic field allows the study of CIDNP-NR at all available fields (0-8 kG

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